FACTORS AFFECTING FIREFLY LARVAL LUMINESCENCE[1]

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The mechanism of control of the adult firefly flash, a burst of light lasting approximately 0.2 second, has been studied intensively (Buck, 1948; McElroy and Hastings, 1955; McElroy and Seliger, 1961; Buck and Case, 1961; Case and Buck, 1963; Buck, Case and Hanson, 1963; Smith, 1963). The larva does not produce a flash, but rather a uniform, structureless glow lasting for seconds (Dahlgren, 1917; Buck, 1948). Histologically its light organs represent a considerably simpler system because they contain no tracheal end cells, cells which are present in the adult light organ and implicated in flash control (Dahlgren, 1917; Snell, 1932; Alexander, 1943).

When an adult firefly is subjected to falling oxygen concentration it remains dark for a short period. Then a dull glow spreads over the organ, gains in intensity, and then slowly declines to extinction. If air is readmitted during this "hypoxic glow" a brilliant pseudoflash is produced, lasting 500 milliseconds or longer (Snell, 1932). The tracheal end cell valve theory of pseudoflash control, proposed by Snell and reaffirmed by Alexander (1943), implied that adult firefly luminescence was normally oxygen-limited and that the pseudoflash was independent of neural activity. Hastings and Buck (1956) also concluded that central nervous activity plays no part in the pseudoflash of the adult. Carlson (1961) examined the pseudoflash of the adult in more detail and implicated neural activity as well as hypoxia.

In lampyrid larvae Buck (1948) and Hastings and Buck (1956) observed that low ambient oxygen induces an hypoxic glow and that subsequently increased oxygen tension elicits a pseudoflash, which resembles that of the adult. The present study of the larval pseudoflash response was initiated to determine in what respect the adult and larval pseudoflash differ. It was hoped the differences in turn could aid in elucidating the disputed role of the adult tracheal end cell in flash control.

Materials and Methods

Larvae of the genus *Photuris* were the subjects of this study. They were collected in the early autumn and stored either in petri dishes on moistened filter paper at 4° C. or in dirt-filled dishes at room temperature. The experimental animal was secured ventral side up on a narrow glass spatula provided with silver stimulating electrodes. The paired stimulating electrodes were usually positioned on each side of the ventral nerve cord in the sixth abdominal segment by insertion

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through the intersegmental membrane between the sixth and seventh abdominal segments. The spatula was placed in the basal segment of a glass Y-tube which was 30 mm. long and 1 cm. in diameter. Oxygen and nitrogen were led into opposite branches of the Y-tube through paired two-way stopcocks which permitted rapid shunting of the oxygen from the animal. Gas mixtures were prepared from commercial compressed nitrogen and oxygen metered through two-stage reduction valves and calibrated Fishcher-Porter flow meters. Composition was checked by gas analysis with a Scholander 0.5-cc. analyzer. Commercial compressed nitrogen, referred to hereafter as "nitrogen," was found to contain no more than 0.05% oxy-

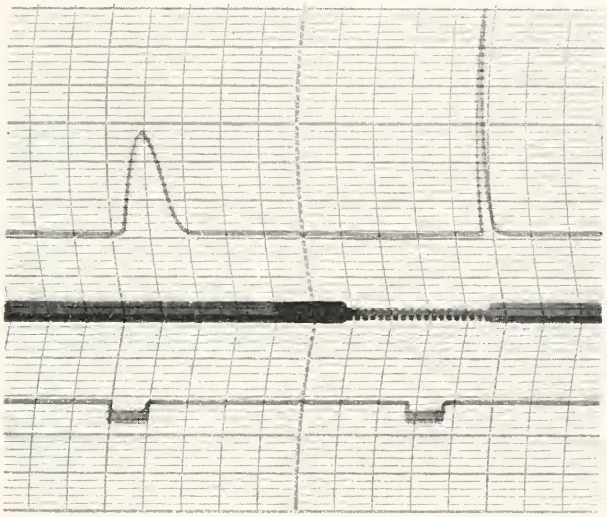

FIGURE 1. Stimulated glow and pseudoflash of *Photuris* larva. In this and all subsequent experiments except where noted: Upper trace is photomultiplier output; middle trace heavy line is 21% oxygen and narrow line is nitrogen; marks on narrow segment are time base, 1 mark per second, reading from left to right. Lower trace: stimulus, 5 volts, 20 msec. duration, 10 per second frequency. Electrode pair inserted in 6th abdominal segment in this and all subsequent experiments of larval light response.

gen which was considered to be a negligible amount. A photomultiplier tube (RCA 931-A) and dissecting microscope were positioned above the animal and both were shielded from stray light by black cloth.

In preparation for a pseudoflash one valve was rotated 180° to shunt off the oxygen and admit either nitrogen or a nitrogen-oxygen mixture. Rotation of this stopcock also opened a signal circuit. At an appropriate time the same valve was then rotated back 180° which allowed a higher concentration of oxygen to reach the animal suddenly and also closed the signal circuit. The pseudoflash was detected by a photomultiplier, the output of which was led to one or both channels of a Grass Polygraph. In some cases the amount of light recorded in a pseudoflash was obtained by integrating the light output with an integrating circuit utilizing a Philbrick operational amplifier.

Results

1. *General characteristics of larval light responses*

The light induced in the larval organ by mechanically irritating the animal is variable in intensity and duration. An electrically stimulated response which mimics the mechanically induced light response is shown in Figure 1. A pseudoflash is also shown. The pseudoflashes of the larval and adult forms resemble each other more closely than do their respective natural light responses, which confirms the observation of Hastings and Buck (1956). The larval pseudoflash differs from the adult pseudoflash, which is illustrated in Figure 2 with a number of spontaneous flashes, in being more variable in duration and considerably longer, due to its increased decay period. Luminescence intensity is uniform over the larval lantern in all light responses.

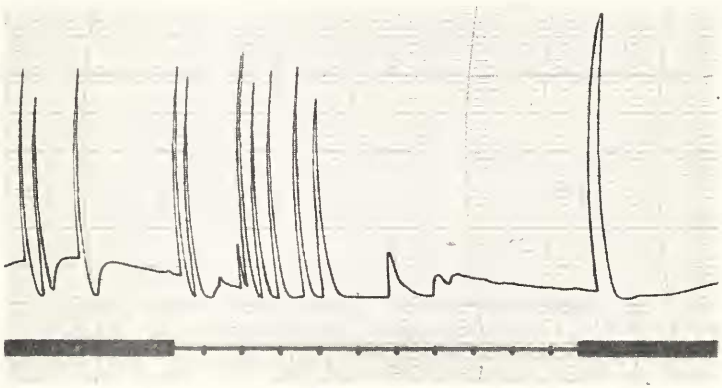

FIGURE 2. Spontaneous flashes and hypoxic glow followed by a pseudoflash in a *Photuris* adult male. Lower trace equivalent to middle trace in Figure 1. Hypoxic glow begins approximately 7 seconds after hypoxic onset; note its relatively low intensity. Note lengthening duration of adult spontaneous flash as anoxia proceeds.

2. *Effect of electrical stimulation and oxygen concentration on larval light responses*

Glow intensity in air is proportional to stimulation frequency up to approximately 10 stimuli per second, above which no further increase can be produced. Induced glow intensity also varies directly with oxygen concentration between 0% and 10% oxygen as shown in Figure 3. Whereas the adult will produce a glow in nitrogen, the larva cannot be induced to glow in this gas after the initial 15 seconds of perfusion. During continual stimulation glows can be maintained for long periods in oxygen concentration as low as 0.25% and the glow level responds to rapid alternation of oxygen concentration.

Larvae that have been glowing actively in air can produce pseudoflashes when the oxygen tension is manipulated. Non-glowing larvae must first be mechanically or electrically stimulated during the anoxic period before a pseudoflash can be produced by admitting oxygen. This stimulation during anoxia need not elicit glowing to be effective in pseudoflash production. Larvae left unstimulated for

periods up to 20 minutes in nitrogen failed to produce a pseudoflash upon readmission of air, but would readily do so if stimulated during the anoxic period.

Like the glow in air, the maximum intensity of the larval pseudoflash is also a function of stimulus frequency. It reaches a maximum intensity at about 10 stimuli

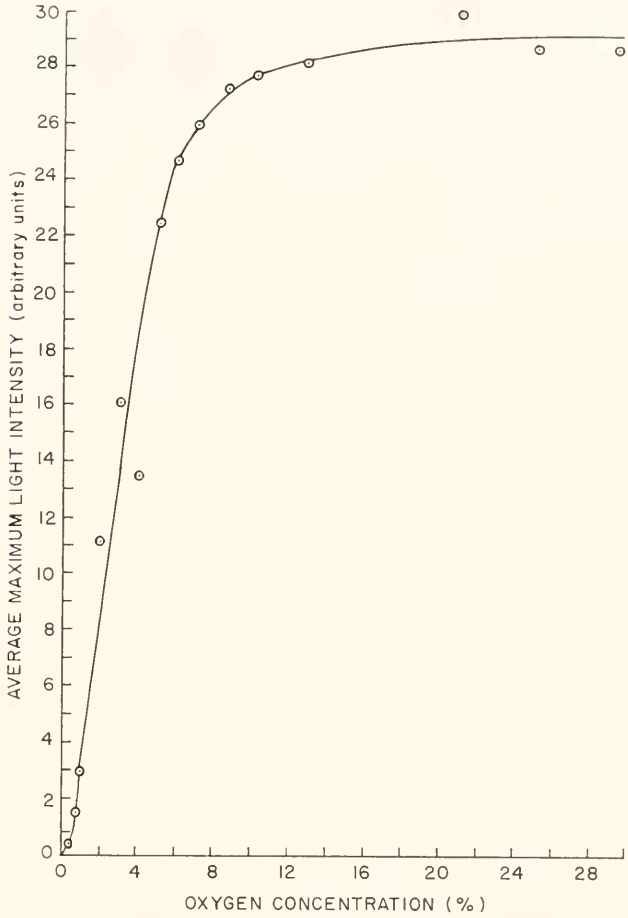

FIGURE 3. Effect of oxygen concentration on the maximum glow intensity attained during stimulation in one *Photuris* larva. Stimulus: 7 volts, 20 msec. duration, 10 per second frequency. Stimulation applied in various oxygen concentrations until a constant, maximum light intensity was attained. Recovery period in air was longer than 60 seconds. Oxygen concentration randomized during repeated experimental runs.

per second when the animal is stimulated during a period in nitrogen, as shown in Figure 4. The total light output of the pseudoflash was closely related to its maximum intensity. If stimulation is continued during readmission of air after hypoxia, an after-glow is produced on the falling phase of the pseudoflash, as shown in Figure 5. Pseudoflash intensity is proportional to oxygen concentrations at least up

to 21% oxygen, as shown in Figure 6. The pseudoflash shape can be changed into a multi-peaked response with rapid alternation of 10% oxygen and nitrogen; see Figure 7.

3. *Effect of hypoxia duration on pseudoflash intensity*

With constant conditions of stimulation, pseudoflash intensity declines as the duration of hypoxia increases, as shown in Figure 8. Long hypoxic durations and

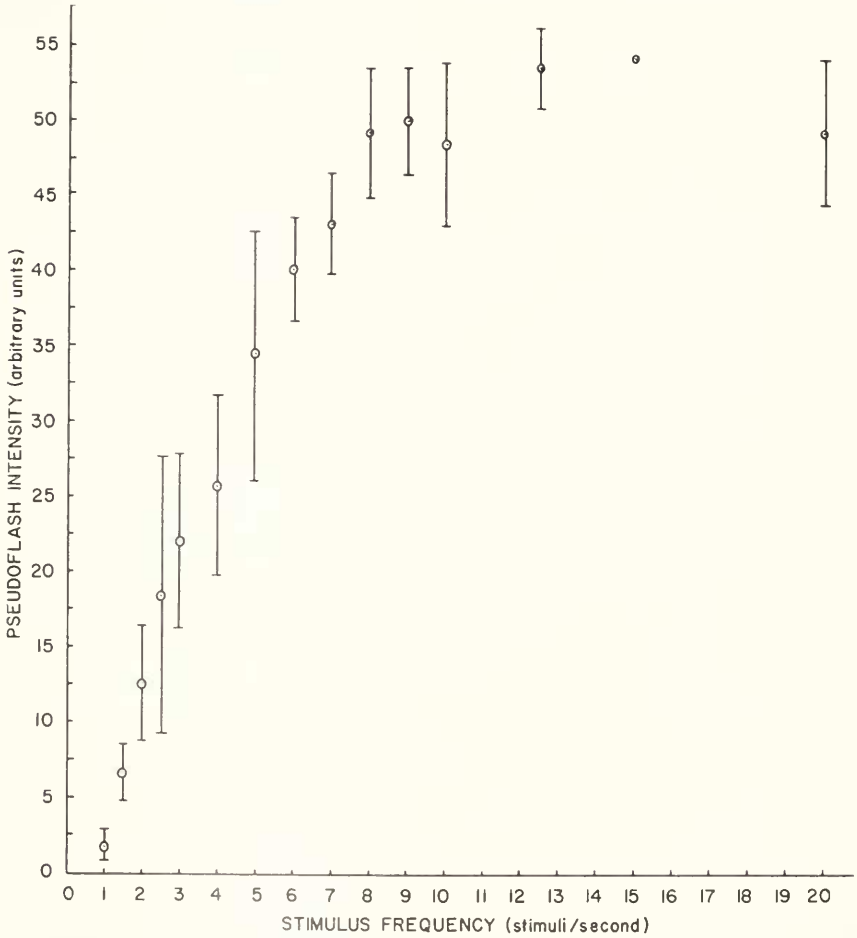

FIGURE 4. Effect of stimulus frequency during anoxia on pseudoflash intensity in *Photuris* larvae. Each point represents the mean pseudoflash intensity of 6 larvae except 2.5 stimuli per second which represents 4 larvae. Bars indicate ± 2 standard errors. All intensities are relative to the mean intensity obtained at 15 stimuli per second in the same individual in order to eliminate differences in geometry of light-collecting system. Stimulus frequency randomized during repeated experimental runs. Stimulus voltage constant for each larva, 20 msec. duration. Stimulation applied 5 seconds after hypoxic onset for a total duration of 5 seconds. Hypoxic duration 15 seconds. Recovery period between pseudoflashes lasted 45 seconds; nitrogen used during hypoxic period and pseudoflashes induced with 21% oxygen.

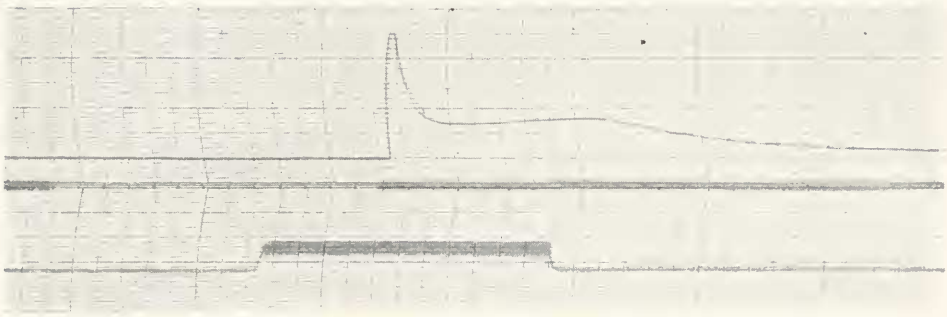

FIGURE 5. Effect of electrical stimulation prior to and during pseudoflash in *Photuris* larva. Lower traces stimulus, 4 volts, 40 msec. duration, 10 per second frequency.

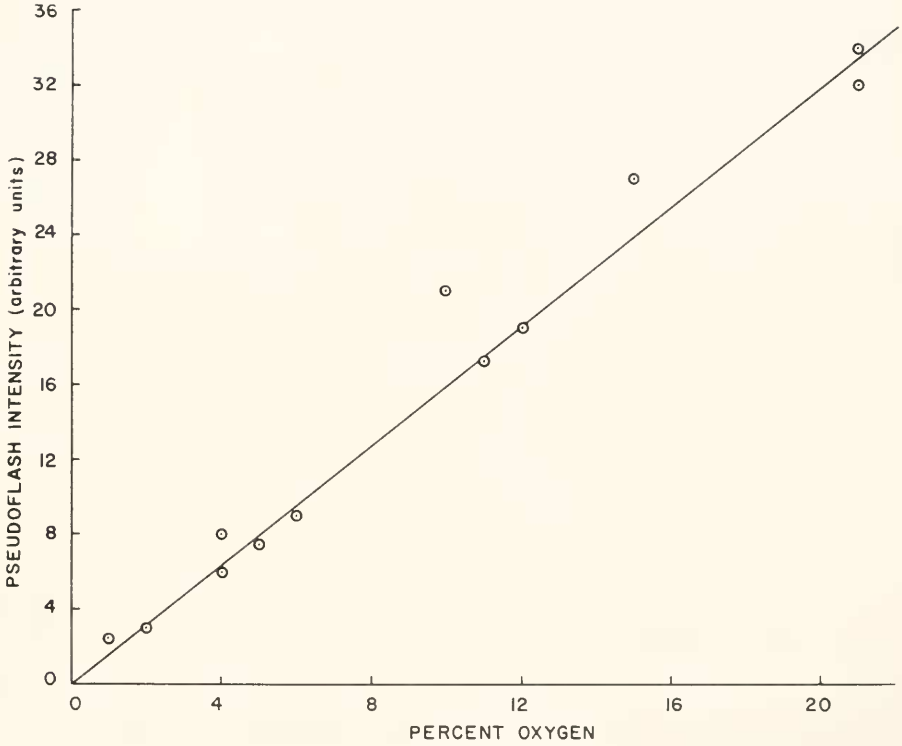

FIGURE 6. Effect of pseudoflash-inducing oxygen concentration on pseudoflash intensity in one *Photuris* larva. Stimulus 3 volts, 20 msec. duration, 10 per second frequency. Stimulation applied 5 seconds after hypoxia onset for a total duration of 5 seconds. Hypoxia duration varied from 16 to 23 seconds. Recovery period between pseudoflashes was 1 to 2 minutes. Nitrogen used during hypoxic period. Oxygen concentration randomized during repeated experimental runs.

their concomitantly reduced pseudoflashes did not affect the intensity of immediately following pseudoflashes induced after short hypoxia.

Discussion

The similarities between the adult and larval pseudoflashes, with respect to induction sequence, response to electrical stimulation and response to oxygen concentration, suggest that both utilize the same basic process. In both developmental forms, the pseudoflash appears to be the result of an accumulation of light-producing substance and its rapid oxidation by the inrushing oxygen.

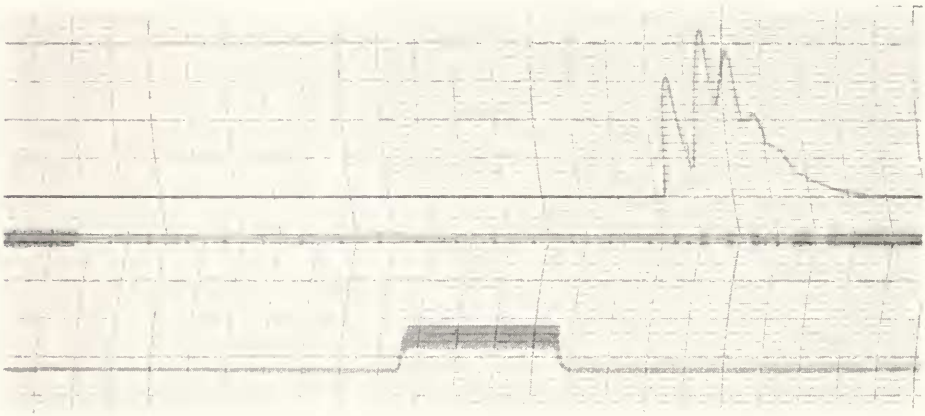

Figure 7. Effect of rapid alternation of 10% oxygen and nitrogen on the pseudoflash response of *Photuris* larva. Middle trace: heavy line, 10% oxygen; narrow line, nitrogen. Lower trace: stimulus, 4 volts, 40 msec. duration, 10 per second frequency.

There are a number of differences, however, between the responses of the two forms.

(1) Adults which are not flashing immediately prior to anoxia can produce a pseudoflash without stimulation during the anoxic period. Non-glowing larvae must first be stimulated in some fashion during anoxia before a pseudoflash can be elicited. This might suggest that it is hypoxia alone which triggers the hypoxic glow in the adult. However, spontaneous neural activity invariably occurs prior to onset of the hypoxic glow in the adult, as observed by Carlson (1962). The need to stimulate the larva then perhaps reflects a relative lack of spontaneous neural activity.

(2) The adult can maintain a glow in nitrogen for several minutes, indicating that oxygen is still available for the light reaction. The larva is apparently completely deoxygenated within 15 seconds in nitrogen because stimulation induces no glow after that period as anoxia continues. However, the anoxic larval photocytes are still responsive to electrical stimulation because stimulation initiated after the first 15 seconds of the anoxic period makes possible a pseudoflash upon readmission of air. This difference in time necessary to flush out the oxygen during exposure to anoxic gas may be a reflection of the relatively more complex tracheal supply of the adult (Buck, 1948). If this assumption is correct the observation

that the larval pseudoflash occupies, on the average, about six times the duration required for the adult pseudoflash cannot be explained on the basis of oxygen diffusion rates to the photogenic tissue. If diffusion rate controlled pseudoflash duration one would expect the adult pseudoflash to be of longer relative duration due to

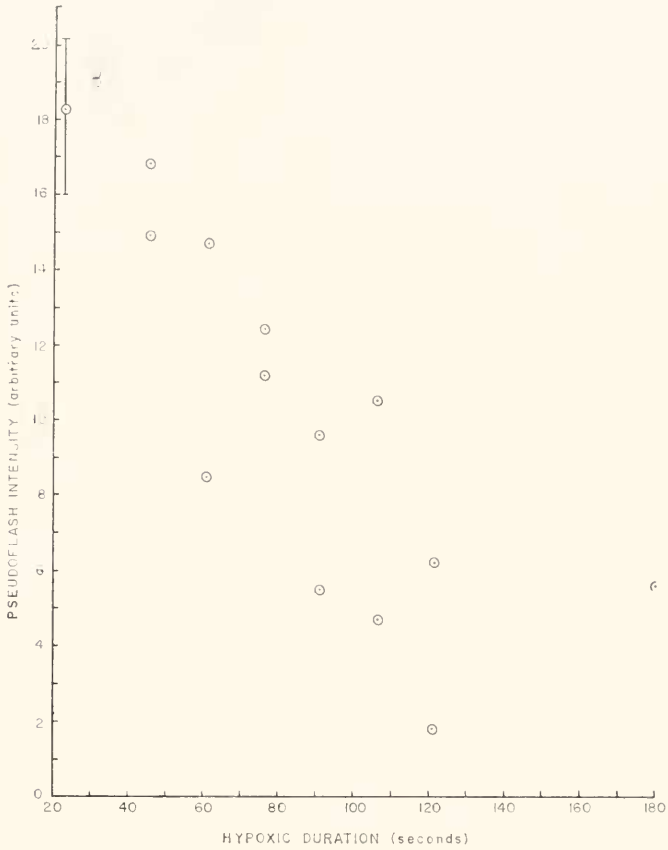

FIGURE 8. Decline of pseudoflash intensity with increasing hypoxia duration under constant conditions of stimulation in one *Photuris* larva. Stimulus: 6 volts, 4 msec. duration and 10 per second frequency. Stimulation applied 15 seconds after hypoxia onset for a total duration of 2 seconds. Recovery period between pseudoflashes was 60 seconds; nitrogen used to produce hypoxic period and pseudoflashes induced with 21% oxygen. First point is average of 34 measurements; line shows total range of values for that point.

the evident impediment to oxygen diffusion noted in its resistance to deoxygenation. The explanation for the differences in pseudoflash duration between the two forms must lie, therefore, at another level in the luminescence process.

(3) As illustrated in Figure 2, as anoxia proceeds the adult spontaneous flashes decline in intensity and increase in duration and then are replaced by a low level glow. Further, this shift from flash response to hypoxic glow fails to develop above an oxygen concentration of about 2.5%; instead the adult can continue to produce

small, spontaneous or electrically driven flashes (Hastings and Buck, 1956). The larval glow shows no such discontinuity but is simply proportional to oxygen concentrations below 10% under uniform stimulus conditions. One might explain these differences on the basis that the larval glow and the adult hypoxic glow are similar phenomena in that they represent processes which are oxygen-limited. Above about 2.0% oxygen, however, another limiting process may be superimposed upon the light reaction in the adult which results in a flash response. This non-oxygen-limiting process may involve the tracheal end cell or may be due to important biochemical differences within the photocytes of the larval and adult forms.

There is no comparable experimental evidence that the inactivation of light-producing substance by non-luminescent means, which may occur in the larva during anoxia, also occurs in the adult. Long anoxic durations, which apparently result in a large inactivation of substance by some dark reaction in the larva, do not prejudice the intensity of later pseudoflashes induced with shorter anoxic periods. It would appear that this non-luminescent inactivation of light-producing substance does not prevent reactivation for use in subsequent flashes.

Summary

1. Electrically stimulated light responses and pseudoflashes were studied in larval fireflies, *Photuris* sp.

2. The larval pseudoflash is highly variable, but it is considerably longer in duration than the pseudoflash produced by the adult.

3. Larvae which were not previously glowing in air would not produce pseudoflashes unless stimulated during the anoxic period prior to admission of oxygen. Even with stimulation no glow could be produced in nitrogen. Pseudoflash intensity is proportional to stimulus frequency up to 10 stimuli per second. Light intensity is dependent on oxygen concentration up to 10% oxygen under uniform stimulus conditions. A multipeaked pseudoflash response can be obtained with rapid alternation of 10% oxygen and nitrogen.

4. Pseudoflash intensity declines with increasing hypoxic duration under uniform stimulus conditions.

5. Differences between the larval and adult pseudoflash response are discussed, but no difference could be linked directly to the operation of the adult tracheal end cell.

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